

## Uptake and Distribution of Manganese and Zinc in *Pinus virginiana* Seedlings Infected with *Pisolithus tinctorius*<sup>1</sup>

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**ABSTRACT.** Nursery grown seedlings of *Pinus virginiana* with infection by *Pisolithus tinctorius* (Pt) versus seedlings with naturally occurring mycorrhizae were used in greenhouse experiments to determine the influence of mycorrhizae on manganese and zinc uptake and distribution in seedlings. Unamended Pt mycorrhizal seedlings and those treated with 48.0 mg Mn/pot over a 2-week period accumulated significantly higher Mn concentrations in shoots than non-Pt mycorrhizal plants. Manganese accumulated in roots of non-Pt mycorrhizal, but not Pt mycorrhizal plants. Seedlings treated with 2.4 mg Zn/pot over a 2-week period had less Mn concentrated in shoots than those unamended treatments. Zinc was significantly more concentrated in the roots than in the shoots of Pt mycorrhizal plants.

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### INTRODUCTION

Survival is difficult for plants in such harsh habitats as strip-mine spoils which lack important nutrients such as nitrogen, phosphorous, and potassium (Corey and Shulte 1973, Lawrey 1977a). Frequently, pH in such soils is extremely low, causing release of metal ions into soil solution (Massey 1972, Berg and Vogel 1973, Loneragan 1975, Lawrey 1977b). Improvement and restoration of surface-mined lands is ultimately dependent upon successful establishment of grasses, forbs, and trees to: (1) stabilize the soil with extensive root systems and addition of organic matter, (2) moderate soil temperatures by shading and water retention, and (3) alter such factors as extreme microclimate and soil conditions to open up niche and habitat space for colonization by new species of plants, animals, and microorganisms.

Pine seedlings with the ectomycorrhizal fungus *Pisolithus tinctorius* (Pt), (Pers.) Coker and Couch, have significantly higher survival rates than those with natural ectomycorrhizae (Marx 1975, 1980, Marx and Artman 1979, Walker et al. 1980). Pine seedlings can become mycorrhizal with naturally-occurring fungi in nursery soils when no special inoculum is used (Marx et al. 1984). However, these seedlings do not exhibit the enhanced growth of Pt mycorrhizal seedlings in mine soils. Thus, trees with particular fungi are adapted to the characteristic environmental conditions of the strip-mine habitat.

Strip-mine reclamation research generally includes analysis of soil chemical conditions such as available nitrogen, phosphorous, and potassium. However, micronutrients such as zinc, copper, manganese, and aluminum receive considerably less attention (Bell and Unger 1981). Some reports of mycorrhizal uptake of heavy metals have been published (Bowen 1973, Berry 1982, Schneck 1982). Toxic effects of heavy metals in plants, which include stunting, chlorophyll destruction, tissue damage, and death, are well known (Foy et al. 1978). High (possibly toxic) concentrations of the metal ions  $Zn^{++}$ ,  $Al^{+++}$ ,  $Mn^{++}$ , and  $Cu^{++}$  in strip-mine spoils make analysis of metal ion toxicity in mycorrhizal seedlings important (Struthers 1964, Massey 1972, Berg and Vogel 1973). In the present study, the effect of infection by the fungal symbiont, *Pisolithus tinctorius*, on uptake of

Mn and Zn in Virginia pine seedlings was examined under controlled greenhouse conditions.

### METHODS AND MATERIALS

One-year-old Virginia pine seedlings, *Pinus virginiana* Mill., were obtained from the Vallonia State Tree Nursery, Indiana Department of Forestry, in October of 1981 (Miller 1983). Nursery beds had been inoculated with *P. tinctorius* in the previous fall (Marx et al. 1984). Seedlings were dug up and transported in coolers from Vallonia to Columbus, Ohio, and were stored in a cold room at slightly above 0°C until potted. Seedlings were placed in ½ gallon (1.89 l.) wax milk cartons approximately ¾ full with a 4:4:4:1 (v/v/v/v) soil mixture of peat, topsoil, perlite, and sand. The soil mixture was well aerated and had low leaching and good cation exchange capacity.

Thirty Pt mycorrhizal and 30 non-Pt-mycorrhizal trees were arranged in a complete block design according to treatment, which involved addition of Mn (as  $MnSO_4$ ), Zn, (as  $ZnSO_4 \cdot 7H_2O$ ) or water to a group of 10 plants. Seedlings were shifted in order to give each block of 10 trees an equal size distribution. All seedlings were watered with deionized water for seven months, and then started on an acidified water treatment of approximately pH 3 which was maintained for the remainder of the study. Metal treatments were started 14 days after watering with acidified water began and were terminated 14 days later. Seedlings were watered with acidified water for another six weeks until harvest.

Soil analysis showed metal ion concentrations from 0.02 ppm to 0.04 ppm for Mn, and 0.01 to 0.05 ppm for Zn. The pH of the soil varied from 7.6 to 8.0. The above values represent both before and after treatment values. Concentrations of metals in treatments were much higher than background soil levels.

Approximately 80 ml of either 5 ppm Zn, 100 ppm Mn, or deionized acidified water were applied to each pot three times a week for the 14-day treatment period. This provided an excess of these trace elements. Total Zn and Mn applied per container was 2.4 mg and 48.0 mg, respectively. Seedling roots probably were not exposed, however, to such concentrations, as soil pH was 7 with some leaching undoubtedly occurring.

All trees were harvested 60 days after application of metals began and air-dried. Mycorrhizal short roots were identified by stereomicroscopic examination, mounted on scanning electron microscope (S.E.M.) stubs, and placed in a desiccator. A scanning electron microscope (Hitachi S-500), with an accelerating voltage of approximately 20,000 V, variable from 10,000 to 30,000 V, was used to perform elemental scanning electron microscopic (SEM) x-ray microanalysis on at least one mycorrhizal section from every seedling (Walker 1979). Thus, at least 10 short roots per treatment were scanned. Shoots and roots were sent to the Ohio Agriculture Research and Development Center (OARDC) for independent elemental analysis. Inductively-coupled plasma analysis (ICP) with an Applied Research Laboratories ICPQ 173 was used to determine tissue Zn and Mn concentrations in roots and shoots (Dahlquist and Knoll 1978).

Soil samples were taken before and after metal treatment. An initial atomic absorption analysis (Lindsey and Norvell 1978) with an atomic absorption spectrophotometer (Perkin Elmer 403) showed low within-group variation (coefficient of variation = 20%). Therefore, soil samples from 10 pots of a given treatment were pooled for analy-

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sis. Each sample was analyzed by atomic absorption spectrometry at the OARDC.

Data were analyzed at the Ohio State University Instructional and Research Computer Center. Two way analysis of variance was conducted with the Statistical Analysis System: PROC ANOVA and GLM (S.A.S. 1979). A Waller-Duncan K-ratio t-test was the post-test performed.

## RESULTS

Pt-infected plants showed varying degrees of Pt mycorrhizal development, whereas the non-Pt-infected seedlings remained free of Pt mycorrhizae. All SEM x-ray microanalysis scans of roots were, however, negative for Mn and Zn. Metals apparently did not accumulate at the parts per hundred thousand level detectable by this method. On the other hand, results of ICP elemental analysis showed significant (ANOVA) differences in shoot metal concentrations of mycorrhizal plants.

Manganese concentration in shoots of greenhouse plants exhibited differences between Pt mycorrhizal and non-Pt mycorrhizal seedlings. Pt mycorrhizal plants had significantly ( $P < 0.05$ , Table 1) higher Mn concentration in shoots than non-Pt mycorrhizal seedlings with or without added Mn, although shoots of both Pt mycorrhizal and non-Pt mycorrhizal plants had increased Mn concentrations. Conversely, Pt mycorrhizal plants treated with Zn had significantly ( $P < 0.05$ ) lower shoot Mn concentrations than the non-Pt mycorrhizal seedlings. Interaction between Zn and Mn also resulted in significantly ( $P < 0.05$ ) lower concentrations of Mn in shoots of Pt mycorrhizal plants than in non-Pt mycorrhizal plants. Interaction between inoculation and metal was significant ( $P < 0.05$ ). Pt mycorrhizal Zn-treated plants had lower Mn shoot concentrations than controls, whereas non-Pt mycorrhizal Zn-treated plants had higher Mn shoot concentrations than controls.

Pt mycorrhizal infection alone resulted in no significant differences in root Mn concentration between mycorrhizal and non-Pt mycorrhizal plants. Manganese concentration was significantly ( $P < 0.05$ ) lower in roots of Mn-treated Pt mycorrhizal plants. Manganese accu-

mulated in non-Pt mycorrhizal roots, but not in Pt mycorrhizal roots.

Zinc distribution in plants was the opposite of that observed for Mn. Zinc concentration in shoots of untreated greenhouse seedlings was not significantly affected by mycorrhizal infection (Table 1). Similarly, treatment with Zn resulted in no significant differences in Zn concentration in shoots of mycorrhizal and non-mycorrhizal plants with or without Mn additions. Untreated plant roots, on the other hand, had a significantly ( $P < 0.05$ , Table 1) greater Zn concentration, when Pt-infected roots were compared to non-Pt-infected roots. Treatment with Zn had the same effect, that is, Pt-infected roots had significantly ( $P < 0.05$ ) greater Zn concentration than non-Pt-infected roots. Seedlings treated with Mn showed no significant difference between Pt-infected and non-Pt-infected roots. For both shoots and roots, the interaction term was not significant, except for the case of the Zn concentration in the shoots of Mn-treated plants ( $P < 0.05$ ). Zinc concentration was less than that of controls in non-Pt infected plants, but the same as controls for Pt-infected plants.

## DISCUSSION

Evolution of heavy metal tolerance in plants has been recognized as an important factor in establishment of vegetation on mined lands (Dykeman and De Sousa 1966, Antonovics, et al. 1971, Simon 1978, Smith and Bradshaw 1979). This tolerance has been correlated with metalliferous sites such as smelters and mines. Antonovics et al. (1971) reported that *Gasteromycetes* grow on mine sites with 20,000 ppm Zn. Selection works on mycorrhizae in the same manner as it does on other organisms (Malloch et al. 1980, Jordon 1981). Ectomycorrhizae of *P. tinctorius* associated with *P. virginiana* must survive the potentially toxic and unfavorable environmental conditions often found on mine sites. In addition, the mycorrhizal symbiont must transfer nutrients from soil solution to host (Vogt et al. 1982). Selection pressure for nutrient absorption for host and fungus

TABLE 1  
Greenhouse Experiments: Metal distribution in Pt-infected and non-Pt-infected pine seedlings.<sup>1</sup>

Mycorrhizal status	Treatment	Plant shoots		Plant roots	
		Mn, ppm $\bar{x} \pm (SE)$	Zn, ppm $\bar{x} \pm (SE)$	Mn, ppm $\bar{x} \pm (SE)$	Zn, ppm $\bar{x} \pm (SE)$
+	Control	275.5* (17.6)	103.0 (16.72)	97.5 (4.5)	71.2* (6.5)
+	Manganese (100 ppm)	327.8* (24.3)	98.1 (6.2)	101.1* (5.2)	57.1 (6.6)
+	Zinc (5 ppm)	243.8 (15.5)	93.6 (8.0)	101.4 (6.4)	91.9* (9.5)
-	Control	202.0 (18.4)	122.5 (18.6)	88.9 (5.6)	47.0 (3.1)
-	Manganese (100 ppm)	247.8 (17.9)	86.5 (10.5)	116.6 (5.4)	60.6 (4.2)
-	Zinc (5 ppm)	259.7 (18.3)	111.6 (8.8)	102.6 (3.8)	63.4 (7.8)

<sup>1</sup>N = 10

\*P < 0.05 Significance level for comparisons between pt-infected and non-pt-infected for each plant part. Two way ANOVA with a Waller Duncan K-ratio t-test for the post-test were the statistical test used.

seems reasonable for sites such as strip-mines, where nutrient availability is often limiting to growth. The fact that sporocarps of *Pisolithus tinctorius*, but not many other fungal species, are found on certain strip-mine sites (Schramm 1966, Medve and Gill 1982, Miller and Rudolph unpublished data 1982), could be due partly to the adaptation of this fungus to high metal concentrations. The adaptation of *P. tinctorius* to temperature extremes (particularly high temperature) is another proposed hypothesis for *Pisolithus* survival on mine sites (Marx and Bryan 1971, Marks and Kozlowski 1973).

The need for the existence of fungal inoculum in soil prior to colonization by various forest species has been hypothesized for at least 15 years (Langford and Buell 1969, Grime 1979). Establishment of *P. tinctorius* mycorrhizal pine seedlings on mined lands appears to be important for successful colonization by certain pine species. The relationship between the fungus and metals, as well as fungus-root-metal interactions, should be considered to determine how this effects reforestation.

Mycorrhizae of *P. tinctorius* in greenhouse-grown seedlings take up significantly more Mn into plants with increased movement to their shoots. Significantly less Mn was found in roots of Pt mycorrhizal than in non-Pt mycorrhizal seedlings. However, in both cases soil Mn concentration was not depleted. Manganese mobilization may be caused by secondary fungal products from Pt mycorrhizae. On the other hand, non-Pt mycorrhizal plants did not show this.

Unlike Mn, Zn uptake and movement into shoots was not affected by Pt infection. This lack of a significant increase in Zn uptake contradicts the findings of other investigators (Bowen et al. 1974, Marx and Artman 1979). However, experimental conditions in these other studies were quite different from the present work. Bowen et al. (1974) used excised roots in liquid culture, whereas Marx and Artman (1979) used field-grown seedlings. Seedlings in the greenhouse may have shown similar results if they were left in contact with the soil solution for a longer time. It was apparent from the ICP analysis that Zn was accumulating in the below ground parts, including possibly the mycorrhizae. The Pt mycorrhizal roots had a significantly greater Zn concentration than non-Pt mycorrhizal roots. Under more acidic soil conditions perhaps the Zn uptake would have been further enhanced. Alternatively, the fungal component may function as an accumulating barrier sequestering the metals, thereby preventing toxic concentrations of Zn from entering roots and thus the plant shoots.

The results from greenhouse-grown seedlings treated with either Zn or Mn, and then analyzed for the other element, generally showed an effect opposite to that found when looking at Zn concentrations resulting from Zn treatments, or Mn concentrations resulting from Mn treatments. This observation points out the possibility of one metal affecting absorption, uptake, and movement of the other. Although these experiments were not designed specifically to study this relationship, it is noteworthy in light of potential metal interactions.

Field data are needed to corroborate the greenhouse and laboratory findings from the present study. Previous field studies have shown differential uptake of metals by mycorrhizal seedlings, when compared to non-my-

corrhizal seedlings (Marx and Artman 1979, Berrent 1981). Additional field studies (under controlled conditions) of a more intensive nature will be necessary, however, to understand mycorrhizae-metal interactions in nature. Research conducted over several growing seasons, for example, would provide a better data base than that obtained over one or two seasons. Comparison of data obtained from disturbed ecosystems such as strip-mined lands with those from undisturbed habitats (Van Hook et al. 1980, Abrahamson and Caswell 1982) will also serve to enhance our understanding of ecosystem processes such as mycorrhizae micronutrient interactions.

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